

A Study in Matrix Interference: The Pierce Quantitative Fluorometric Peptide Assay and the SUMS Proteomics Landscape

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ThermoFisher Scientific's Pierce Quantitative Fluorometric Peptide Assay kit is an indispensable component of our bottom-up proteomics sample preparation workflow at SUMS. We use this assay to determine peptide recovery efficiency for our various proteomics methods.

However, per the Pierce kit's technical note, the assay's fluorescence is susceptible to matrix interference from organic solvents, volatile salts, etc. While the individual effects of interfering agents are well-studied, their combined effects are as yet unknown. This uncertainty poses significant implications for our routine proteomics analyses because users' biosample matrices vary extensively depending on the specifics of their experiment. Therefore, it is critical to understand the impact of typical matrices on our peptide quantification assays.

Hence, this project aims at probing the cumulative effects of different matrix components on the accuracy of the fluorometric peptide assay. We will screen several of our routine peptide elution solutions, as well as real-world peptide extracts from user projects to verify the sources of variability. These will be compared to a relatively simple and clean control matrix, consisting of 2% acetonitrile in water with 0.1% formic acid.

Our findings aim to demystify the effects of complex matrix interferents on quantitative bottom-up proteomics experiments and enable us, and other core facilities, to better rely on such assays, and understand their limits.